Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Biochemical isolation and characterization of glucose-6-phosphate dehydrogenase, 6-Phosphogluconate dehydrogenase and glutathione reductase enzymes from camel kidney

Mohamed M. Abdel-Monsef*, Doaa A. Darwish, Mahmoud A. Ibrahim

Molecular Biology Department, National Research Centre, Postal code: 12622, El-Tahrir st., Dokki, Giza, Egypt.

Key words: 6-phosphogluconate dehydrogenase, Camel kidney, Glucose-6-phosphate dehydrogenase, Glutathione reductase.

***Corresponding Author: Mohamed M. Abdel-Monsef,** Molecular Biology Department, National Research Centre, Postal code: 12622, El-Tahrir st., Dokki, Giza, Egypt.

Abstract

A rapid and simple purification method of glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase (6PGD) and glutathione reductase (GR) from camel kidney with a high yield was carried out. Camel kidney enzymes (G6PD, 6PGD, and GR) were purified homogeneously by one chromatographic step on 2′, 5′ ADP Sepharose 4B affinity column. After chromatography, the specific activity of G6PD was increased to 19.5 units/mg protein with 65.4% yield and 300-fold purification, while the specific activity of 6PGD was increased to 42.4 units/mg protein with 75.8% yield and 557.9-fold purification and the specific activity of GR was increased to 35.5 units/mg protein with 68.9% yield and 710-fold purification. G6PD, 6PGD and GR enzymes are appeared homogeneous on both native and 12% SDS-PAGE, with molecular weights of 60 kDa, 58 kDa and 65 kDa respectively. They displayed their optimum activity at pH 8.0, 8.2 and 7.8 respectively. The divalent cations $MgCl_2$ and $MnCl_2$ increased G6PD and 6PGD activity, while $FeCl_2$, $CuCl_2$ and $ZnCl_2$ inhibited them. $CuCl_2$, $ZnCl_2$ and $NiCl_2$ increased GR activity, while $FeCl_2$ and $MgCl_2$ inhibited it. NADPH inhibited G6PD and 6PGD activity while NADP inhibited GR activity. This study is the first report on the purification of G6PD, 6PGD, and GR from the camel kidney. A task for the future is the production of these enzymes for industrial medical applications.

Introduction

Glucose 6-phosphate dehydrogenase (G6PD) is the key and first enzyme of the pentose phosphate metabolic pathway that catalyzes the glucose 6-phosphate conversion to 6-phosphogluconate in the presence of $NADP^+$. There are two main tasks of the pentose phosphate pathway in the cells, ribose 5-phosphate synthesis that is required for DNA, RNA and ribonucleotides and synthesis of phosphorylated carbohydrates that are important for synthesis of aromatic amino acids, vitamins and sedoheptulose-7-phosphate [1]. G6PD was purified from different mammalian organisms and tissues including rabbit liver [2], camel liver [3], mouse liver [4], dog liver [5], sheep erythrocytes [6], sheep kidney [7], rat kidney [8], from grass carp [1] and from bacteria [9]. 6-Phosphogluconate dehydrogenase (6PGD) is the second enzyme of the pentose phosphate metabolic pathway that catalyzes the transformation of 6-phosphogluconate to D-ribulose-5-phosphate and reduces NADP to NADPH [10]. 6PGD can be considered as an antioxidant enzyme due to yielding of NADPH, which protects the cell against oxidant agents by yielding reduced glutathione (GSH) and shares in the production of some molecules as fatty acids, steroids and amino acids [11, 12, 13]. The reactions catalyzed by G6PD and 6PGD in the pentose phosphate metabolic pathway are considered the major source of NADPH [12]. 6PGD enzyme has been purified from various sources from bacteria to mammals as rat erythrocytes [10], rat lung [14], chicken liver [15], from bacteria [16] and from grass carp [17]. Glutathione reductase (GR) is a member of the pyridine-nucleotide disulfide oxidoreductase family of flavoenzymes that performs a critical part to maintain a high intracellular GSH/GSSG ratio and converts NADPH to $NADP^+$ [18, 19]. GR has been purified from numerous various origins as diatom algae [20], rainbow trout liver [21], bovine erythrocytes [22], bovine liver [23], sheep liver [24, 25] and turkey liver [26]. The pentose phosphate pathway cycle is major for tumor cell metabolism and growth. G6PD deficiency is one of the haemolytic anaemia reasons and inhibitors of 6PGD and GR are used as anticancer and antimalarial drugs [27, 28]. Knowing the behaviour of these enzymes and their inhibitors will be helpful in the drug design and pharmaceutical investigations. Therefore, this study aims at purification and characterization of three important antioxidant enzymes G6PD, 6PGD and GR from the

camel kidney using affinity chromatography. Such a study will be helpful to understand the regulatory mechanisms of these enzymes. The camel kidney is very large organ, available, safe, cheap and rich source for extraction and purification of G6PD, 6PGD and GR enzymes in comparison with the other sources and will allow large scale production of the three enzymes. G6PD enzyme is used in the preparation of glucose diagnostic kit (HK/G6PD; hexokinase/glucose-6-phosphate dehydrogenase) which is used for in vitro quantitative determination of glucose for diagnostic and medical purposes. G6PD, 6PGD and GR enzymes are included in antioxidant drugs preparations to protect the skin and hair against inflammatory reactions associated with chemical irritation.

Material and methods

Kidney tissues

Six fresh kidney samples of healthy adult camels *Camelus dromedarius* were obtained from a local governmental slaughter-house in Cairo and preserved at -20 °C. The weights of kidneys were 900-1000 gm, and the dimensions were 9-10 cm width and 15-17 cm length. The health of camels was inspected by slaughter house veterinary doctors before slaughtering.

Chemicals

Glucose-6-phosphate (G6P), 6-phosphogluconic acid trisodium salt (6PG), β-Nicotinamide adenine dinucleotide phosphate ($NADP^+$), β-Nicotinamide adenine dinucleotide phosphate, reduced (NADPH) and all the fine chemicals were purchased from Sigma-Aldrich Chemical Co. (Germany). Molecular weight SDS marker proteins and 2′, 5′ ADP Sepharose 4B were products of GE Healthcare Fine Chemicals Co. (Sweden). The other chemicals were of analytical grade and purchased from Carl Roth Chemical Co. (Germany).

Assays of G6PD, 6PGD and GR enzymes activities

The G6PD assay reaction mixture contained in 1 ml 100 mM Tris-HC1 buffer pH 8.0, containing 0.2 mM NADP, 10 mM $MgCl_2$ and 0.6 mM G6P. The reaction was started by enzyme addition and G6PD activity monitored by detecting the rate of NADPH production level spectrophotometrically at 340 nm [29]. The 6PGD assay reaction mixture contained in 1 ml 50 mM Tris-HC1 buffer pH 7.5, containing 3.3 mM $MgC1_2$, 0.25 mM NADP and 2 mM 6-phosphogluconate. The reaction was started by enzyme addition and 6PGD activity monitored by detecting the rate of NADPH production level at wavelength 340 nm [30]. One unit of G6PD or 6PGD was defined as the amount of enzyme catalyzing the reduction of 1 μmol of NADP/min. The GR assay reaction mixture contained in a total volume of 1 ml, 50 mM K-phosphate buffer, pH 7.0, 1 mM EDTA, 0.1 mM NADPH, 0.5 mM oxidized glutathione and the enzyme solution. One unit of GR activity is defined as the amount of enzyme which oxidizes 1 μ mole of NADPH per min [31].

G6PD, 6PGD and GR activity staining on polyacrylamide gels

For G6PD activity staining, the gel was submerged in 40 ml of 0.1 M Tris-HCI buffer pH 8, containing 4 mg of Phenyl methosulfate (PMS), 3.2 mg of Nitroblue tetrazolium (NBT), 4 mg of $MgCI_2$, 13 mg of glucose 6-phosphate. The gels were incubated in the staining solution in dark at 37 °C for 60-90 min [32]. For 6PGD activity staining, the gel was submerged in 40 ml of 0.1 M Tris-HCI buffer pH 8, containing 4 mg of PMS, 3.2 mg of NBT, 4 mg of $MgCI_2$, 13 mg of 6-phosphogluconate trisodium salt. The gels were incubated in the staining solution in dark at 37 °C for 60 min [33]. For GR activity staining, the gel was soaked in 25 ml of 50 mM Tris-HCl buffer, pH 7.9, containing 4.0 mM GSSG, 1.5 mM b-NADPH, and 2 mM DTNB with gentle shaking for 20 min. After a brief rinse with 50 mM Tris-HCl buffer pH 7.9, the GR activity was negatively stained in darkness by 50 ml 1.2 mM MTT and 1.6 mM PMS for 5–10 min at room temperature with gentle shaking. Clear zones of GR activity against the purple background were found [34].

Purification of G6PD, 6PGD and GR from camel kidney

Preparation of crude extract

All of the procedures were performed at 4 ºC unless stated otherwise. Ten grams of fresh kidney were minced and homogenized by omni-mixer (Sorvall Dupont Instruments), with two volumes (2 ml/gm tissue) of 0.02 M K-phosphate buffer, pH 7.6 containing 1 mM DTT and 1 mM EDTA on ice. The mixture was centrifuged at 12.000 xg for 30 min at 4 ˚C and the pellet was discarded. The supernatant was saved and designated the crude extract.

Affinity chromatography on 2′, 5′ ADP Sepharose 4B column

2 gm of 2′, 5′ ADP Sepharose 4B resin was suspended in 200 ml distilled water. The gel was degassed, suspended in 0.1 M K-phosphate buffer, pH 6.0, packed in the column (10 x 1 cm) and equilibrated with 0.05 M K-phosphate buffer including 1 mM DTT, 1 mM EDTA, pH 7.5, by means of a peristaltic pump. The flow rate was adjusted to 20 ml/h. The sample was loaded onto the column and washed with 25 ml of 0.1 M K-phosphate, pH 6 and 50 ml of 0.1 M K-phosphate, pH 7.8, containing 0.1 M NaCl. Then, the washing was continued with 0.05 M K-phosphate buffer including 1 mM EDTA, 1 mM DTT, pH 7.5, until the absorbance became zero at 280

nm. The G6PD enzyme was eluted with 100 ml of 0.1 M K-phosphate buffer pH 7.8 containing 80 mM NaCl, 0.5 mM NADP and 1 mM EDTA. After elution of G6PD activity, the 6PGD enzyme was eluted with 100 ml of 0.1 M K-phosphate buffer pH 7.8 containing 80 mM NaCl, 5.0 mM NADP and 1 mM EDTA. After elution of 6PGD activity, the GR enzyme was eluted with 100 ml 0.1 M K-phosphate buffer pH 7.8 containing 1 mM GSH, 0.5 mM NADPH and 1 mM EDTA [8]. The activities of all fractions were measured and graphed.

Electrophoretic analysis

Native gel electrophoresis was carried out using 7% PAGE according to Smith [35]. SDS-PAGE was performed with 12% polyacrylamide gel according to Laemmli [36]. The molecular weights of the purified G6PD, 6PGD and GR enzymes were determined using SDS-PAGE as described by Weber and Osborn [37]. Proteins were stained with 0.25% coomassie brilliant blue R-250.

Protein determination

Protein was quantified by the dye binding assay method using bovine serum albumin (BSA) as a standard protein [38].

Results and discussion

Purification of G6PD, 6PGD and GR from camel kidney

The major aim of this study is to establish a high-yield and fast procedure for the purification to homogeneity of the three antioxidant enzymes G6PD, 6PGD and GR. Here, a fast, simple and very convenient purification method for G6PD, 6PGD and GR from camel kidney by one chromatographic step on 2′, 5′ ADP Sepharose 4B affinity column was carried out. The purification scheme of G6PD, 6PGD and GR is summarized in (Table 1). After chromatography on 2′, 5′ ADP Sepharose 4B column (Figure 1), G6PD specific activity was increased to 19.5 units/mg protein representing 65.4% yield and 300-fold purification over the crude extract, 6PGD specific activity was increased to 42.4 units/mg protein representing 75.8% yield and 557.9-fold purification over the crude extract and GR specific activity was increased to 35.5 units/mg protein representing 68.9% yield and 710-fold purification over the crude extract (Table 1). Different specific activities and purification folds have been stated for these enzymes. G6PD from sheep kidney had a specific activity of 27.69 units/mg protein, 1348-fold purification and 16.96% yield [2] and from sheep erythrocytes had a specific activity of 4.6 units/mg protein and 37.1% yield [6]. 6PGD from rat erythrocytes had a specific activity of 5.15 units/mg protein, 2575-fold purification and 78.4% yield [10], 6PGD from chicken liver had a specific activity of 61 units/mg protein, 344-fold purification and 5.5% yield [15] and from grass carp with a specific activity of 5.2 units/mg protein, 309-fold purification and 68% yield [17]. GR from human erythrocyte had a specific activity of 20.7 units/mg protein, 8600-fold purification and 26% yield [18]. GR from bovine liver has been purified 5456-fold purification and 38.4% yield [23], from rainbow trout liver had a specific activity of 27.7 units/mg protein, 1654-fold purification and 41% yield [21] and from turkey liver had a specific activity of 606.6 units/mg protein, 2476-fold purification and 10.7% yield [26].

Table 1. A typical purification scheme of the purified camel kidney G6PD, 6PGD and GR enzymes.

Purification steps*	Total protein (mg)	Total Activity (unit†)	Specific Activity‡	Yield (%)	Fold Purification
G6PD					
Crude extract	395	25.7	0.065	100	1.0
2′, 5′ ADP Sepharose 4B Affinity chromatography	0.86	16.8	19.5	65.4	300.0
6PGD					
Crude extract	395	30.2	0.076	100	1.0
2′, 5′ ADP Sepharose 4B Affinity chromatography	0.54	22.9	42.4	75.8	557.9
GR					
Crude extract	395	19.6	0.05	100	1.0
2′, 5′ ADP Sepharose 4B Affinity chromatography	0.38	13.5	35.5	68.9	710.0

†One unit of G6PD or 6PGD enzymes activity is identified as the amount of enzyme required to reduce 1 μmol of $NADP^+$ per minute.
†One unit of GR enzyme activity is identified as the amount of enzyme required to oxidize 1 μmol of NADPH per minute.
‡Specific activity is expressed as units/mg protein.

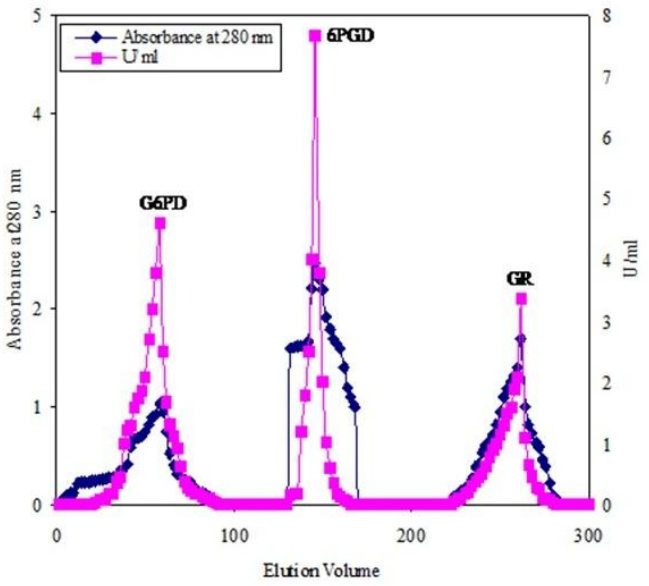

Figure 1. A typical elution profile for the chromatography of the camel kidney crude extract on 2′, 5′ ADP sepharose 4B column (1 cm x 10 cm) previously equilibrated with 0.05M K-phosphate buffer including 1 mM DTT, 1 mM EDTA, pH 7.5. The G6PD enzyme was eluted with 0.1 M K-phosphate buffer pH 7.8 containing 80 mM NaCl, 0.5 mM NADP and 1 mM EDTA, the 6PGD enzyme was eluted with 0.1 M K-phosphate buffer pH 7.8 containing 80 mM NaCl, 5.0 mM NADP and1 mM EDTA and the GR enzyme was eluted with 0.1 M K-phosphate buffer pH 7.8 containing 1 mM GSH, 0.5 mM NADPH and 1 mM EDTA. 2 ml fractions were collected at a flow rate of 20 ml/hour.

Electrophoretic analysis of G6PD, 6PGD and GR

The purity of G6PD, 6PGD and GR eluted from the 2′, 5′ ADP Sepharose 4B column was tested by analysis on 7% native PAGE. The three enzymes G6PD, 6PGD and GR showed one protein band in each (Figure 2a) corresponding the isoenzymes activity bands (Figure 3) revealed a homogeneous preparation and indicating the purity of these enzymes. The molecular weights of G6PD, 6PGD and GR were determined by SDS-PAGE to be 60 ± 1.22, 58 ± 1.43 and 65 ± 1.37 kDa respectively (Figure 2b). Similar molecular weights were reported; 62 kDa of bovine lens G6PD [39], 59 kDa of rat erythrocytes 6PGD [10], 64 kDa of sheep brain GR [40] and 55 kDa of bovine liver GR [23] while diatom algae GR was 118 kDa [20].

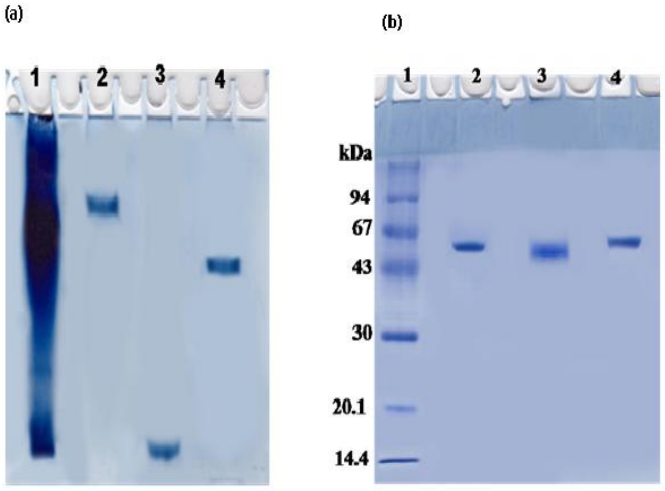

Figure 2. (a) Electrophoretic analysis of camel kidney G6PD, 6PGD and GR protein patterns on 7% native PAGE, (1) crude extract, (2) 2′, 5′ ADP sepharose 4B fractions of G6PD, (3). 2′, 5′ ADP Sepharose 4B fractions of 6PGD and (4) 2′, 5′ ADP Sepharose 4B fractions of GR. (b) Molecular weight determination by electrophoretic analysis of G6PD, 6PGD and GR on 12% SDS-PAGE, (1) molecular weight marker proteins, (2) purified G6PD, (3) purified 6PGD and (4) purified GR.

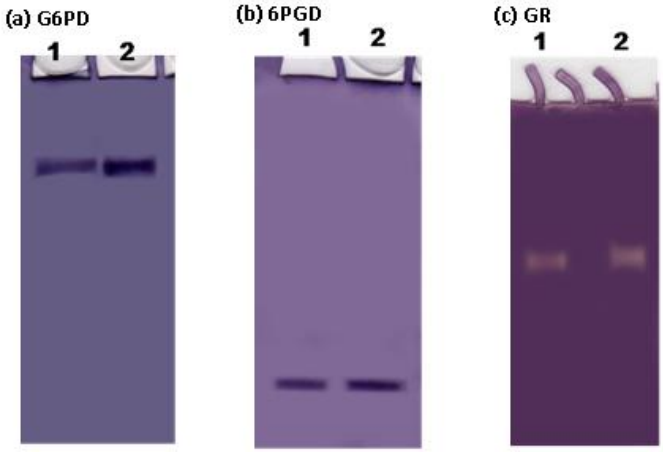

Figure 3. Electrophoretic analysis of camel kidney G6PD, 6PGD and GR isoenzyme patterns on 7% native PAGE, (a) G6PD isoenzyme pattern: (1) crude extract and (2) 2′, 5′ ADP Sepharose 4B fraction. (b) 6PGD isoenzyme pattern: (1) crude extract and (2) 2′, 5′ ADP sepharose 4B fraction. (c) GR isoenzyme pattern: (1) crude extract and (2) 2′, 5′ ADP Sepharose 4B fraction.

Determination of optimum pH

The effect of pH on the purified G6PD, 6PGD and GR activities were examined in 0.02 M K-phosphate buffer, pH (7.2 - 9.0). The pH profile of G6PD, 6PGD and GR displayed their optimum activities at pH 8.0, 8.2 and 7.8 respectively Figure (4). Similar pH optimum ranges were reported to be 7.8 for lamb kidney G6PD [19], 8.0 for rat erythrocytes 6PGD [10], 8.0 for diatom algae GR [20] and 7.0 for bovine liver GR [23].

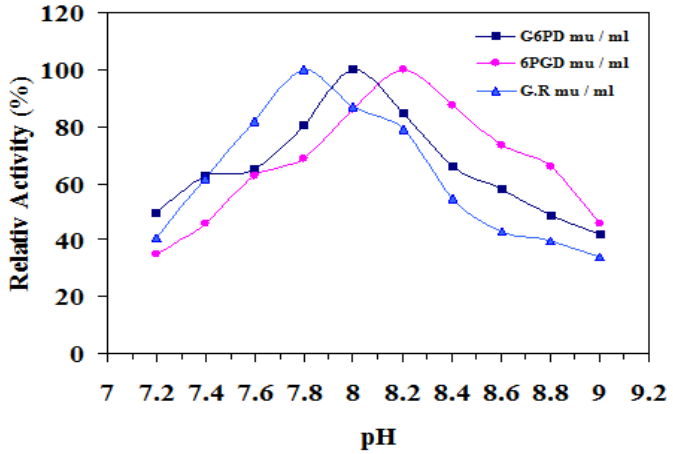

Figure 4. Effect of pH on the purified G6PD, 6PGD and GR using 0.02 M K-phosphate buffer of various pH values.

Effect of divalent cations on the purified camel kidney G6PD, 6PGD and GR enzymes

The purified G6PD, 6PGD and GR enzymes were preincubated with 2 mM and 5 mM of each cation for 5 min at 37 ˚C and the activity was assayed. A control test without any cation was taken as 100% relative activity. The activity of G6PD was inhibited in presence of $FeCl_2$, $CuCl_2$ and $ZnCl_2$ while increased with $MnCl_2$ and $MgCl_2$ (Table 2), this is in good agreement with G6PD of rainbow trout liver [41]. Also, 6PGD was inhibited in presence of $FeCl_2$, $CuCl_2$ and $ZnCl_2$ and increased with $MnCl_2$ and $MgCl_2$. While GR activity increased by $CuCl_2$, $ZnCl_2$ and $NiCl_2$ and inhibited by $FeCl_2$ and $MgCl_2$ in agreement with rainbow trout liver GR [21].

Effect of various inhibitors on the purified camel kidney G6PD, 6PGD and GR enzymes

The purified G6PD, 6PGD and GR enzymes were preincubated with 2 mM and 5 mM of each inhibitor (0.1 mM and 0.2 mM of NADPH for G6PD and 6PGD and 0.1 mM and 0.2 mM of NADP for GR) for 5 min at 37 ˚C and the residual activity was calculated as a ratio of a control lacking inhibitor (Table 3).

Table 2. Effect of divalent cations on the purified camel kidney G6PD, 6PGD and GR enzymes.

Reagent	Final Concentration (mM)	Residual activity (%)		
		G6PD	6PGD	GR
Control	-----	100.0	100.0	100.0
$CoCl_2$	2.0	90.2	96.5	87.0
	5.0	88.3	92.7	96.4
$MnCl_2$	2.0	114	110	95.3
	5.0	119	135	89.2
$FeCl_2$	2.0	39.2	35.1	78.3
	5.0	31.3	29.5	54.6
$ZnCl_2$	2.0	49.8	68.0	118
	5.0	41.1	60.2	177
$CuCl_2$	2.0	25.3	53.5	116
	5.0	20.5	41.8	122
$NiCl_2$	2.0	99.8	94.3	110
	5.0	99.1	93.1	130
$MgCl_2$	2.0	145	133	85.0
	5.0	178	169	79.2
$CaCl_2$	2.0	90.2	96.5	87.0
	5.0	89.0	92.7	80.1

Table 3. Effect of various inhibitors on camel kidney G6PD, 6PGD and GR enzymes.

Reagent	Final Conc. (mM)	Inhibition (%)			Residual activity		
		G6PD	6PGD	GR	G6PD	6PGD	GR
Control	-----	0	0	0	100	100	100
NADPH	0.1	76.8	67.9	0	23.2	32.1	100
	0.2	89.7	94.2	0	10.3	5.80	100
NADP	0.1	0	0	80.1	100	100	19.9
	0.2	0	0	94.3	100	100	5.70
1,10 Phenanthroline	2.0	13.2	9.90	15.4	86.8	90.1	84.6
	5.0	15.3	1.11	17.6	84.7	89.8	82.4
Phenyl methyl sulfonyl fluoride (PMSF)	2.0	30.4	33.7	40.1	69.6	66.3	59.9
	5.0	35.5	39.6	45.7	64.5	60.4	54.3
Iodoacetamide	2.0	5.12	11.7	18.6	94.8	88.3	81.4
	5.0	7.95	12.8	19.9	92.0	87.2	80.1
β-Mercaptoethanol	2.0	40.8	61.9	39.4	59.2	38.1	60.6
	5.0	50.7	69.7	43.5	49.7	30.3	56.5
Dithiothreitol (DTT)	2.0	51.3	41.7	60.1	48.7	58.3	39.9
	5.0	59.4	50.8	66.7	40.6	49.2	33.3
EDTA	2.0	8.23	5.17	0.27	91.7	94.8	99.7
	5.0	9.45	8.42	0.45	90.5	91.6	99.5
ADP	2.0	20.1	22.6	10.1	79.9	77.4	89.9
	5.0	25.7	29.8	14.6	74.3	70.2	85.4
ATP	2.0	0.25	0.50	5.77	99.7	99.5	94.2
	5.0	2.43	2.78	6.69	97.6	97.2	93.3
NAD	2.0	0	0	70.1	100	100	29.9
	5.0	0	0	75.9	100	100	24.1
NADH	2.0	22.6	20.3	0	77.4	79.7	100
	5.0	37.4	35.2	0	62.6	64.8	100

NADPH is found to be the most potent inhibitor for the G6PD and 6PGD activities, while NADP is the most potent inhibitor of GR activity. ATP and EDTA were found to be very weak inhibitors for all enzymes. 1, 10 Phenanthroline and ADP are weak inhibitors for all enzymes. β-Mercaptoethanol, DTT and PMSF are moderate inhibitors for all enzyme activity. NAD has no inhibitory effect on G6PD and 6PGD but inhibited GR activity. β-Mercaptoethanol and DTT inhibited G6PD, 6PGD and GR indicating that SH groups in the active site of these enzymes important for their activity. PMSF inhibited G6PD, 6PGD and GR indicating that serine is involved in the active site of these enzymes.

Conclusion

In Conclusion, this study presents a simple, rapid and convenient method for the purification of G6PD, 6PGD and GR from camel kidney with a high recovery. The procedure is adaptable for enzymes production by scaling up; the characterization of the purified enzymes will allow their uses with maximum efficiency.

Acknowledgements

This work was funded by the National Research Centre, Egypt.

Conflict of interest

The authors declare that there are no conflicts of interest. All authors have approved the manuscript and agree with its submission to the journal of applied pharmaceutical science.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors. We get our samples from slaughtered camel in slaughter-house.

Abbreviations

G6PD: Glucose-6-phosphate dehydrogenase, 6PGD: 6-Phosphogluconate dehydrogenase, GR: Glutathione reductase, G6P: Glucose-6-phosphate, GSSG: Oxidized glutathione, GSH: Reduced glutathione, $NADP^{+:}$ β-

Nicotinamide adenine dinucleotide phosphate, NADPH: Reduced β-Nicotinamide adenine dinucleotide phosphate, NBT: Nitroblue tetrazolium, PAGE: Polyacrylamide gel electrophoresis, PMS: Phenazine methosulfate, ROS: Reactive oxygen species, MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, DTNB: 5,5′-Dithiobis(2-nitrobenzoic acid).

References

1. Hu W, Zhi L, Zhuo MQ, Zhu QL, Zheng JL, Chen QL, Gong Y, Liu CX. Purification and characterization of glucose 6-phosphate dehydrogenase (G6PD) from grass carp (*Ctenopharyngodon idella*) and inhibition effects of several metal ions on G6PD activity in vitro. Fish Physiol Biochem. 2013; 39(3):637-647.
2. Ulusu NN, Tandogan B. Purification and kinetics of sheep kidney cortex glucose-6-phosphate dehydrogenase. Comp Biochem Physiol. 2006; 143: 249-255.
3. Ibrahim MA, Ghazy AM, Salem AMH, Ghazy MA, Abdel-Monsef MM. Purification and characterization of glucose-6-phosphate dehydrogenase from camel liver. Enz Res, 2014, 1-10. Doi: http://dx.doi.org/10.1155/2014/714054.
4. Ozols J. Isolation and complete amino acid sequence of luminal endoplasmic reticulum glucose-6-phosphate dehydrogenase. Proc Natl Acad Sci USA 1993; 90: 5302-5306.
5. Velasco P, Barcia R, Ibarguren I, Sieiro MA, Ramos-martinez JI. Purification, characterization and kinetic mechanism of glucose-6-phosphate dehydrogenase from mouse liver. Int J Biochem. 1994; 26: 195-200.
6. Beydemir S, Ciftçi M, Küfrevioğlu OI. Purification and characterization of glucose 6-phosphate dehydrogenase from sheep erythrocytes and inhibitory effects of some antibiotics on enzyme activity. J Enzyme Inhib Med Chem. 2002; 17(4): 271-277.
7. Özer N, Bilgi C, Ögüsa I H. Dog liver glucose-6-phosphate dehydrogenase: purification and kinetic properties. Int J Biochem Cell Biol. 2002; 34: 253-262.
8. Adem S, Ciftci M. Purification of rat kidney glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and glutathione reductase enzymes using 2′, 5′ ADP Sepharose 4B affinity in a single chromatography step. Prot Exp Pur. 2012; 81: 1- 4.
9. Acero-Navarro KE, Jiménez-Ramírez M, Villalobos MA, Vargas-Martínez R, Perales-Vela HV, Velasco-García R. Cloning, overexpression, and purification of glucose-6-phosphate dehydrogenase of *Pseudomonas aeruginosa*. Prot Expr Purif. 2018; 142: 53-61.
10. Beydemir S, Ciftci M, Yilmaz H, Bakan E Kufrevioglu OI. 6-Phosphogluconate dehydrogenase: Purification, characterization and kinetic properties from rat erythrocytes. Turk J Vet Anim Sci. 2004; 28: 707-714.
11. Kozar RA, Weibel CJ, Cipolla J, Klein AJ, Haber MM, Abedin MZ, Trooskin SZ. Antioxidant enzymes are induced during recovery from acute lung injury. Crit Care Med. 2000; 28: 2486-2491.
12. Lehninger AL, Nelson DL, Cox MM. 2000. Principles of Biochemistry. New York., 2nd ed. Worth Publishers Inc. 558-560.
13. Bianchi D, Bertrand O, Haupt K, Coello N. Effect of gluconic acid as a secondary carbon source on non-growing L-lysine producer cells of *Corynebacterium glutamicum*. Purification and properties of 6-phosphogluconate dehydrogenase. Enzy Microb Technol. 2001; 28: 754-759.
14. Adem S, Ciftci M. Purification and biochemical characterization of glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and glutathione reductase from rat lung and inhibition effects of some antibiotics. J Enzyme Inhib Med Chem. 2016; 31: 1342-1348.
15. Erat M. Purification of 6-phosphogluconate dehydrogenase from chicken liver and investigation of some kinetic properties. Prep Biochem Biotechnol. 2005; 35: 53-69.
16. Wang Y, Zhang P. Over expression and simple purification of the Thermotoga maritima 6-phosphogluconate dehydrogenase in *Escherichia coli* and its application for NADPH regeneration. Microbial Cell Factories 2009; 4: 8-30.
17. Sun L, Luo Z, Hu W, Zhuo M, Zheng, J, Chen Q, Liang, X, Xiong B. Purification and characterization of 6-phosphogluconate dehydrogenase (6-PGD) from grass carp (*Ctenopharyngodon idella*) hepatopancreas. Indian J Biochem Biophys. 2013; 50: 554-561.
18. Erat M. Purification of human erythrocyte glucose-6-phosphate dehydrogenase and glutathione reductase enzymes using 2′, 5′ ADP Sepharose 4B affinity column material in single chromatographic step. Prot Exp Pur. 2004; 34: 257-260.
19. Ulusu NN, Tandogan B, Tezcan FE. Kinetic properties of glucose-6-phosphate dehydrogenase from lamb kidney cortex. Biochimie. 2005a; 87: 187-190.
20. Arias DG,Marquez VE, Beccaria AJ, Guerrero SA, Iglesias AA. Purification and characterization of a glutathione reductase from *Phaeodactylum tricornutum*. Protist. 2010; 161: 91-101.
21. Tekam B, Ozdemir H, Senturk M, Ciftci M. Purification and characterization of glutathione reductase from rainbow trout (*Oncorhynchus mykiss*) liver and inhibition effects of metal ions on enzyme activity. Comp Biochem Physiol Toxicol Pharm. 2008; 148: 117-121.
22. Erat M, Sakiroglu H, Ciftci M. Purification and characterization of glutathione reductase from bovine erythrocytes. Prep Biochem Biotechnol. 2003; 33: 283-300.
23. 23. Ulusu NN, Tandogan B. Purification and kinetic properties of glutathione reductase from bovine liver. Mol Cell Biochem, 2007; 303: 45-51.
24. Erat M, Ciftci M. In vitro effects of some antibiotics on glutathione reductase from sheep liver. J Enzyme Inhib Med Chem. 2003; 18: 545-550.
25. Ulusu G, Erat M, Ciftci M, Sakuroulu H, Bakan E. Purification and characterization of glutathione reductase from sheep liver. Turk J Vet Anim Sci. 2005b; 29: 1109-1117.
26. Taser P, Ciftci M. Purification and characterization of glutathione reductase from turkey liver. Turk J Vet Anim Sci. 2012; 36: 546-553.
27. Beutler E, Duparc S. Glucose-6-phosphate dehydrogenase deficiency and antimalarial drug development. Amer J Trop Med Hyg. 2007; 77: 779-789.
28. Elf S, Lin R, Xia S, Pan Y, Shan C, Wu S, Lonial S, Gaddh M, Arellano ML, Khoury HJ, Khuri FR, Lee BH, Boggon TJ, Fan J, Chen J. Targeting 6-phosphogluconate dehydrogenase in the oxidative PPP sensitizes leukemia cells to antimalarial agent dihydroartemisinin. Oncogene. 2017; 36(2): 254-262.
29. Betke K, Brewer GJ, Kirkman HN, Luzzato L, Motulsky AG, Ramot B, Siniscalco M. Standardized method for G-6-PD assay of haemolysates. WHO Tech Rep Ser. 1967; 366: 30-32.
30. Medina-puerta MD, Garrido-pertierra A. Purification and properties of the enzyme 6-phosphogluconate dehydrogenase from *Dicentrarchus labrax* L. liver. Comp Biochem Physiol. 1986; 83: 215-220.
31. Zanetti G. Rabbit liver glutathione reductase: Purification and properties. Arch Biochem Biophys. 1979; 198 (1): 241-246.
32. Rodriguez-segad S F M. Partial purification and properties of three enzymatic forms of glucose 6-phosphate dehydrogenase from *mytilus edulis* L. hepatopancreas. Comp Biochem Physiol. 1982; 72: 117-121.
33. Smith A. Low erythrocyte glucose-6-phosphate dehydrogenase activity and primaquine insensitivity in sheep. J Lab Clin Med. 1968; 71: 826-830.
34. Hou WC, Liang HJ, Wang CC, Liu DZ. Detection of glutathione reductase after electrophoresis on native or sodium dodecyl sulfate polyacrylamide gels. Electrophoresis 2004; 25: 2926-2931.
35. Smith I. Acrylamide gel disc electrophoresis in "Electrophoretic techniques". New York, Academic press 1969.
36. Laemmli UK. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. Nature 1970; 227: 680-685.
37. Weber K, Osborn M. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J Biol Chem. 1969; 244: 4406-4412.
38. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976; 72: 248-254.
39. Ulusu NN, Kus MS, Acan NL, Tezcan EF. A rapid method for the purification of glucose-6-phosphate dehydrogenase from bovine lens. Int J Biochem Cell Biol. 1999; 31: 787-796.
40. Acan NL, Tezcan EF. Sheep brain glutathione reductase: purification and general properties. FEBS Lett. 1989; 250: 72-74.
41. Cankaya M, Sisecioglu M, Ciftci M, Ozdemir H. Effects of Some Metal Ions on Trout Liver Glucose 6-phosphate Dehydrogenase. Res J Environ Toxicol. 2011; 5(6): 385-391.